

Cellular immune responses in porcine reproductive and respiratory syndrome virus (PRRSV) vaccinated weaned piglets challenged with a virulent strain of PRRSV

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Abstract

The porcine reproductive and respiratory syndrome (PRRS) is an important viral infection of swine that can persist in lymphoid organs of infected pigs despite the induction of specific immune responses, suggesting an immune evasion mechanism. Vaccination has been shown to prevent clinical signs but remains ineffective against viral persistence. Our objective was to investigate the immunological disorders related to vaccination and subsequent challenge by a virulent strain. Groups of piglets were vaccinated with RespPRRS vaccine (Boehringer-Ingelheim) and challenged 4 weeks later with the virulent LHVA-93-3 strain of the PRRS virus. Animals were sacrificed at various times after vaccination and the lungs and lymphoid organs were collected. Lymphoid cell subsets were analysed and viral persistency was determined by RT-PCR. No modifications were observed in the percentages of CD2+CD4+ and CD2+CD8^{high} T cells in PRRSV-vaccinated animals, whereas these cells decreased in spleen, tonsils and lymph nodes in vaccinated-challenged piglets. Similarly, specific antibody secreting-B cells were increased after vaccination, but decreased following challenge with the virulent virus. Persistent viral RNA was found in lungs, blood, tonsils, and lymph nodes up to 24 days either in vaccinated or vaccinated-challenged pigs. Taken together, these results indicate that vaccination favours a decrease in CD4+ and CD8 cells and antibody producing-B cells in blood and lymphoid organs and viral persistency in animals challenged with a virulent virus, suggesting a failure in the immune processes.

Introduction

Porcine reproductive and respiratory syndrome virus (PRRSV) represents a widespread viral infection which remains uncontrolled in many countries despite the use of attenuated vaccines. These vaccines decrease the lesions in lungs and significantly improve reproductive performance of the sow (1). However, different vaccinal strains express different immunological properties, thus conferring different levels of protection in pigs (2). In this respect, the clinical protection and the immune responsiveness to PRRSV infections varies considerably between farms and sows within farms (3). The PRRSV is known to persist during several weeks in lungs and lymphoid organs (4). We have previously demonstrated that, in spite of a transient polyclonal activation of B cells, the metabolic activity of lymphoid cells from blood and spleen decreased up to 60 days p.i., while specific anti-PRRSV-secreting B cells occurred later in blood and lymphoid organs (5). In addition, the percentages of the CD2+CD8^{high} cell subset increased in spleen and blood of experimentally-PRRSV-infected pigs up to 45 days while they did not increase in mediastinal lymph nodes (MLN) and tonsils (6). However, lymphoid T cells isolated from the blood or lymphoid organs of PRRSV persistently-infected pigs were hyporesponsive to mitogens, an effect correlating with viral replication (6).

Recently, Diaz et al. (2) confirmed the absence of neutralizing antibodies and low frequencies of virus-specific IFN- γ secreting cells in pigs vaccinated with two European-type modified live strains. These observations suggest that attenuated live vaccines can induce immunodisorders favouring viral persistence in absence of clinical signs.

In this work, we report that vaccination with an attenuated PRRSV strain does not prevent viral persistence and correlates with decreased numbers of $CD4^+$ and $CD8^{high}$ cells and of antibody producing-B cells in blood and lymphoid organs from animals challenged with a virulent virus variant.

Materials and methods

Virus and experimental infection in pigs: Forty young pigs were vaccinated with RespPRRS vaccine (Boehringer-Ingelheim) and challenged, 4 weeks later with the virulent strain LHVA-93-3 of the PRRS virus. At various times after vaccination (p.v.) or challenge (p.c.) animals were sacrificed and lungs and lymphoid organs were collected.

Lymphoid cells: The lymphocytes were isolated from blood, spleen, mediastinal lymph nodes (MLN) and tonsils as previously described (5). Lymphoid cells were incubated with anti-CD2 mAb (Cedarlane; Hornby, Ontario, Canada) and anti-CD4 mAb (VMRD Inc. Pullman, WA) or anti-CD8 mAb (Cedarlane) and then with secondary antibodies: phycoerythrin (PE)-conjugated anti-rat IgG2a; (Cedarlane) and fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG2a (Cedarlane). Flow cytometric analysis was done using a FACScan (Becton-Dickinson, Mountain View, CA) and a Cell Quest software (Becton-Dickinson). Ten thousand events were analyzed per sample and the percentages of $CD2^+CD4^+$, $CD2^+CD8^{high}$, and cell subsets were determined by multiparametric analysis (6). Specific antibody-secreting B cells were evaluated by an ELISPOT assay, and virus detection by done by RT-PCR as reported previously (5).

Statistical analysis:

The difference in the percentages of cell subsets measured by flow cytometric in unvaccinated and PRRSV-vaccinated animals or vaccinated-challenged animals at each time p.i. were evaluated using a Student's t test. A probability level of $P < 0.05$ was considered significant.

Results

Viral RNA was detected in tonsils, spleen and MLN as soon as three days p.i. while it was detected after 10 days in lungs of most of vaccinated animals and persisted up to 24 days in lungs and lymphoid organs. Following challenge with a virulent PRRSV strain, viral RNA increase at three days p.c. and persisted up to 24 days in most animals (Table 1).

To verify the relationship between lymphoid T cell subsets and viral persistence in lymphoid organs, the percentages of $CD2^+CD4^+$ and $CD2^+CD8^{high}$ T cell populations were studied in blood, spleen, MLN and tonsils of PRRSV-vaccinated and vaccinated-challenged weaned pigs at various times. As shown in figure 1A, the percentages of $CD2^+CD4^+$ T cells were not altered in blood, spleen, tonsils and MLN in vaccinated pigs (except in tonsils at 24 days p.v.) ($p < 0.05$). However $CD2^+CD4^+$ cells decreased in the blood and lymphoid organs three days after challenge, and two weeks later in blood and MLN. In addition, the percentages of $CD2^+CD8^{high}$ decreased 10 days pi in blood and MLN after vaccinated-challenged pigs ($p < 0.05$) (Fig. 1B).

The production of specific antibody secreting-B cells in blood was stimulated in vaccinated animals at 10 days p.v. and later in lymphoid organs (Fig. 2). Surprisingly, the number of secreting-B cells decreased two weeks after challenge with virulent PRRSV in blood, spleen and MLN. It was not possible to evaluate the number of specific antibody secreting-B cells in tonsils since bacterial infections occurred 10 days after the challenge.

Discussion

The use of live vaccine against PRRSV infection in weaned pigs induced no abnormalities in $CD4^+$ and $CD8^{high}$ T cells and stimulated the production of B-secreting antiviral antibodies. However, following challenge with a virulent PRRSV strain the percentages of $CD4^+$ cells and $CD8^{high}$ T cells, and specific B-secreting cells decreased while viral RNA persisted in the lungs and lymphoid organs of most animals.

Organs	Vaccinated (days post vaccination)				Vaccinated-challenged (days post challenge)			
	3	10	17	24	3	10	17	24
Lungs	0/4	3/4	3/4	3/4	3/4	3/4	4/4	3/4
Tonsils	3/4	3/4	3/4	3/4	4/4	4/4	4/4	4/4
MLN	3/4	3/4	2/4	2/4	3/4	4/4	4/4	3/4
Spleen	3/4	2/4	2/4	2/4	3/4	3/4	3/4	2/4

Table 1: Detection of viral RNA in lungs and lymphoid organs from vaccinated and vaccinated-challenged pigs. Viral RNA was detected by a RT-PCR method.

A) CD2+CD4+

B) CD2+CD8^{high}

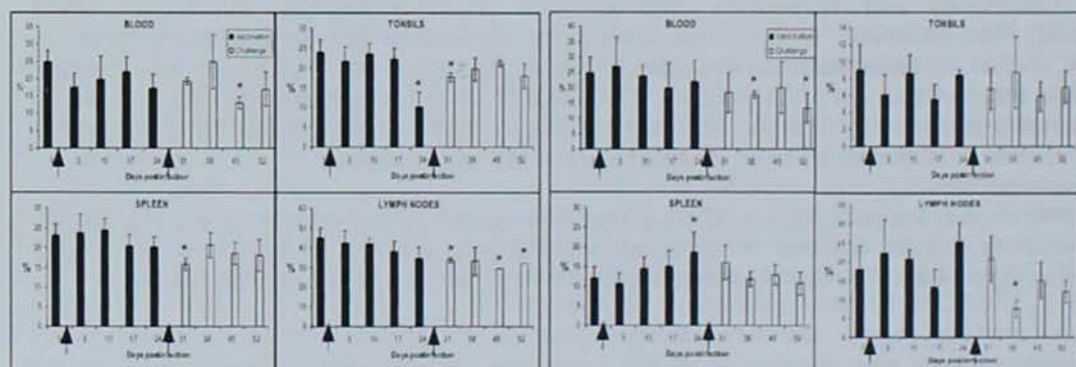


Figure 1: Percentages of CD2+CD4+ (A) and CD2+CD8^{high} (B) in blood, spleen, tonsils and mediastinal lymph nodes from PRRSV vaccinated and vaccinated-challenged pigs.* p<0.05

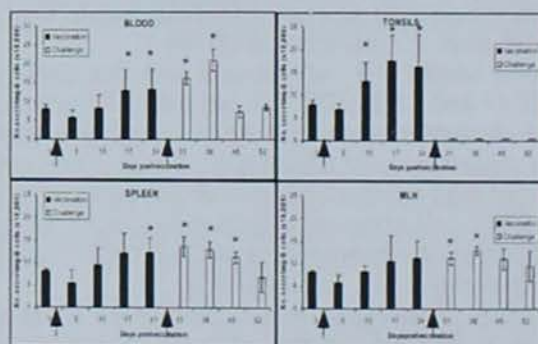


Figure 2: Specific anti-PRRSV antibody secreting-B cells in tonsils, spleen, mediastinal lymph nodes, and blood from vaccinated and vaccinated-challenged pigs.* p<0.05

These observations are in accordance with previous studies which have reported no significant changes in CD4 and CD8 cells subsets in pigs vaccinated with a modified live virus (7). The decreased percentages in CD4+ and CD8+ T cells and in specific antibody secreting-B cells in blood, spleen and MLN observed in vaccinated-challenged animals suggests that a generalized immunosuppression may occur, or that CD4+, CD8^{high} and B cells are recruited to infected tonsils

or lungs. The simultaneous decrease of T and B cell subsets and persistence of viral RNA in lungs and lymphoid organs observed in vaccinated-challenged pigs support the first hypothesis. However, we have previously shown that the virulent PRRSV strain used for the challenge did not significantly modify the percentages of CD4+ cells in spleen, MLN or tonsils in the first days p.i. suggesting that the cell immunosuppression does not directly result from the effects of challenging virus (6). Kiss *et al.* (8) have observed that new variant PRRS viruses might originate from interaction with virulent and vaccine viral strains in vaccinated herds showing recurrence of the disease. Recent works also suggest that PRRSV infection increased the production of suppressive cytokines, such as IL-10 (9). We can propose that the virus used for challenge or new generated variants may exacerbate the production of immunosuppressive cytokines in vaccinated animal due to the viral persistence in macrophages from lungs and lymphoid organs. The second hypothesis concerning a viral-induced recruitment of antiviral T and B cells in infected organs is not supported by our results, since no significant increase in lymphoid cells were taken place in the tonsils in spite of the fact that lymphoid cells in the lungs were not included in this study. In addition, viral RNA was present in spleen and MLN.

Taken together, these results indicate that vaccination does not prevent viral persistence but rather favours a decrease in CD4+ and CD8 T cells and in antibody producing-B cells in blood and lymphoid organs when animals are subsequently infected with a virulent virus, suggesting a failure in the immune processes.

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